=> d l5 ibib abs 1-8

L5 ANSWER 1 OF 8 CAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 2002:553063 CAPLUS
DOCUMENT NUMBER: 137:88460
TITLE: Use of **JAQ1** (monoclonal **antibody anti GPVI**) as a drug for the protection against thrombotic diseases
INVENTOR(S): **Nieswandt, Bernhard**
PATENT ASSIGNEE(S): Germany
SOURCE: Eur. Pat. Appl., 24 pp.
CODEN: EPXXDW
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
EP 1224942	A1	20020724	EP 2001-101406	20010123
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO, MK, CY, AL, TR				
EP 1228768	A1	20020807	EP 2001-130953	20011228
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO, MK, CY, AL, TR				

PRIORITY APPLN. INFO.: EP 2001-101406 A 20010123
AB A medicament for the protection against thrombotic diseases is described that comprises an active principle which induces irreversible inactivation or degrdn. of the collagen receptor on thrombocytes. Antibodies, esp. the humanized monoclonal antibody **JAQ1**, are the preferred active principle. Further a diagnostic agent for the detn. of the expression rate of the collagen receptor **GPVI** is disclosed which contains the labeled monoclonal or polyclonal **antibody** directed against the **GPVI** epitope, preferably as defined by **JAQ1**.
REFERENCE COUNT: 8 THERE ARE 8 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L5 ANSWER 2 OF 8 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.DUPLICATE 1
ACCESSION NUMBER: 2001:425540 BIOSIS
DOCUMENT NUMBER: PREV200100425540
TITLE: A novel viper venom metalloproteinase, alborhagin, is an agonist at the platelet collagen receptor GPVI.
AUTHOR(S): Andrews, Robert K. (1); Gardiner, Elizabeth E.; Asazuma, Naoki; Berlanga, Oscar; Tulasne, David; **Nieswandt, Bernhard**; Smith, A. Ian; Berndt, Michael C.; Watson, Stephen P.
CORPORATE SOURCE: (1) Baker Medical Research Inst., St. Kilda Rd. Central, Melbourne, VIC, 8008: rkandrews@hotmail.com Australia
SOURCE: Journal of Biological Chemistry, (July 27, 2001) Vol. 276, No. 30, pp. 28092-28097. print.
ISSN: 0021-9258.
DOCUMENT TYPE: Article
LANGUAGE: English
SUMMARY LANGUAGE: English
AB The interaction of platelet membrane glycoprotein VI (**GPVI**) with collagen can initiate (patho)physiological thrombus formation. The viper venom C-type lectin family proteins convulxin and alboaggregin-A activate platelets by interacting with **GPVI**. In this study, we isolated from white-lipped tree viper (Trimeresurus albolabris) venom, alborhagin, which is functionally related to convulxin because it activates platelets but is structurally different and related to venom metalloproteinases. Alborhagin-induced platelet aggregation (EC50, <7.5 mug/ml) was inhibitable by an **anti**-alphaIIbbeta3 **antibody**, CRC64,

and the Src family kinase inhibitor PP1, suggesting that alborhagin activates platelets, leading to alphaIIbbeta3-dependent aggregation. Additional evidence suggested that, like convulxin, alborhagin activated platelets by a mechanism involving **GPVI**. First, alborhagin- and convulxin-treated platelets showed a similar tyrosine phosphorylation pattern, including a similar level of phospholipase Cgamma2 phosphorylation. Second, alborhagin induced **GPVI**-dependent responses in **GPVI**-transfected K562 and Jurkat cells. Third, alborhagin-dependent aggregation of mouse platelets was inhibited by the **anti-GPVI** monoclonal **antibody JAQ1**.
Alborhagin had minimal effect on convulxin binding to **GPVI** -expressing cells, indicating that these venom proteins may recognize distinct binding sites. Characterization of alborhagin as a **GPVI** agonist that is structurally distinct from convulxin demonstrates the versatility of snake venom toxins and provides a novel probe for **GPVI**-dependent platelet activation.

L5 ANSWER 3 OF 8 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.DUPLICATE 2
ACCESSION NUMBER: 2001:324006 BIOSIS
DOCUMENT NUMBER: PREV200100324006
TITLE: Evidence for cross-talk between glycoprotein VI and Gi-coupled receptors during collagen-induced platelet aggregation.
AUTHOR(S): **Nieswandt, Bernhard (1)**; Bergmeier, Wolfgang; Eckly, Anita; Schulte, Valerie; Ohlmann, Philippe; Cazenave, Jean-Pierre; Zirngibl, Hubert; Offermanns, Stefan; Gachet, Christian
CORPORATE SOURCE: (1) Ferdinand-Sauerbruch Klinikum Wuppertal, Witten/Herdecke University, Arrenbergerstrasse 20, Haus 10, 42117, Wuppertal: nieswand@klinikum-wuppertal.de Germany
SOURCE: Blood, (June 15, 2001) Vol. 97, No. 12, pp. 3829-3835. print.
ISSN: 0006-4971.
DOCUMENT TYPE: Article
LANGUAGE: English
SUMMARY LANGUAGE: English
AB Collagen-induced platelet aggregation is a complex process and involves synergistic action of integrins, immunoglobulin (Ig)-like receptors, G-protein-coupled receptors and their ligands, most importantly collagen itself, thromboxane A2 (TXA2), and adenosine diphosphate (ADP). The precise role of each of these receptor systems in the overall processes of activation and aggregation, however, is still poorly defined. Among the collagen receptors expressed on platelets, glycoprotein (GP) VI has been identified to play a crucial role in collagen-induced activation. **GPVI** is associated with the FcRgamma chain, which serves as the signal transducing unit of the receptor complex. It is well known that clustering of **GPVI** by highly specific agonists results in platelet activation and irreversible aggregation, but it is unclear whether collagen has the same effect on the receptor. This study shows that platelets from Galphaq-deficient mice, despite their severely impaired response to collagen, normally aggregate on clustering of **GPVI**, suggesting this not to be the principal mechanism by which collagen activates platelets. On the other hand, dimerization of **GPVI** by a monoclonal **antibody** (**JAQ1**), which by itself did not induce aggregation, provided a sufficient stimulus to potentiate platelet responses to Gi-coupled, but not Gq-coupled, agonists. The combination of **JAQ1** and adrenaline or ADP, but not serotonin, resulted in alphaIIbbeta3-dependent aggregation that occurred without intracellular calcium mobilization and shape change in the absence of Galphaq or the P2Y1 receptor. Together, these results provide evidence for a cross-talk between (dimerized) **GPVI** and Gi-coupled receptors during collagen-induced platelet aggregation.

L5 ANSWER 4 OF 8 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.DUPLICATE 3

ACCESSION NUMBER: 2001:418811 BIOSIS
DOCUMENT NUMBER: PREV200100418811
TITLE: Platelet glycoprotein V binds to collagen and participates in platelet adhesion and aggregation.
AUTHOR(S): Moog, Sylvie; Mangin, Pierre; Lenain, Nadege; Strassel, Catherine; Ravanat, Catherine; Schuhler, Simone; Freund, Monique; Santer, Martine; Kahn, Mark; **Nieswandt, Bernhard**; Gachet, Christian; Cazenave, Jean-Pierre; Lanza, Francois (1)
CORPORATE SOURCE: (1) INSERM U.311, 10 Rue Spielmann, Etablissement Francais du Sang-Alsace, 67065, Strasbourg Cedex: francois.lanza@efs-alsace.fr France
SOURCE: Blood, (August 15, 2001) Vol. 98, No. 4, pp. 1038-1046. print.
ISSN: 0006-4971.
DOCUMENT TYPE: Article
LANGUAGE: English
SUMMARY LANGUAGE: English
AB Glycoprotein V (GPV) is a subunit of the platelet GPIb-V-IX receptor for von Wille-brand factor and thrombin. GPV is cleaved from the platelet surface during activation by thrombin, but its role in hemostasis is still unknown. It is reported that GPV knockout mice had a decreased tendency to form arterial occluding thrombi in an intravital thrombosis model and abnormal platelet interaction with the subendothelium. In vitro, GPV-deficient platelets exhibited defective adhesion to a collagen type I-coated surface under flow or static conditions. Aggregation studies demonstrated a decreased response of the GPV-deficient platelets to collagen, reflected by an increased lag phase and reduced amplitude of aggregation. Responses to adenosine diphosphate, arachidonic acid, and the thromboxane analog U46619 were normal but were enhanced to low thrombin concentrations. The defect of GPV null platelets made them more sensitive to inhibition by the **anti-GPVI** monoclonal **antibody** (mAb) **JAQ1**, and this was also the case in aspirin- or apyrase-treated platelets. Moreover, an mAb (V.3) against the extracellular domain of human GPV selectively inhibited collagen-induced aggregation in human or rat platelets. V.3 injected in rats as a bolus decreased the ex vivo collagen aggregation response without affecting the platelet count. Finally, surface plasmon resonance studies demonstrated binding of recombinant soluble GPV on a collagen-coupled matrix. In conclusion, GPV binds to collagen and appears to be required for normal platelet responses to this agonist.

L5 ANSWER 5 OF 8 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.DUPLICATE 4
ACCESSION NUMBER: 2001:242500 BIOSIS
DOCUMENT NUMBER: PREV200100242500
TITLE: Long-term antithrombotic protection by in vivo depletion of platelet glycoprotein VI in mice.
AUTHOR(S): **Nieswandt, Bernhard (1)**; Schulte, Valerie; Bergmeier, Wolfgang; Mokhtari-Nejad, Rabee; Rackebrandt, Kirsten; Cazenave, Jean-Pierre; Ohlmann, Philippe; Gachet, Christian; Zirngibl, Hubert
CORPORATE SOURCE: (1) Ferdinand Sauerbruch Klinikum Elberfeld, Universitaet Witten/Herdecke, Arrenbergerstr. 20, Haus 10, D-42117, Wuppertal: nieswand@klinikum-wuppertal.de Germany
SOURCE: Journal of Experimental Medicine, (February 19, 2001) Vol. 193, No. 4, pp. 459-469. print.
ISSN: 0022-1007.
DOCUMENT TYPE: Article
LANGUAGE: English
SUMMARY LANGUAGE: English
AB Coronary artery thrombosis is often initiated by abrupt disruption of the atherosclerotic plaque and activation of platelets on the subendothelial layers in the disrupted plaque. The extracellular matrix protein collagen is the most thrombogenic constituent of the subendothelial layer;

therefore, a selective inhibition of the collagen activation pathway in platelets may provide strong antithrombotic protection while preserving other platelet functions. Here we demonstrate that treatment of mice with a monoclonal **antibody** against the activating platelet collagen receptor glycoprotein VI (**GPVI**; **JAQ1**) results in specific depletion of the receptor from circulating platelets and abolished responses of these cells to collagen and collagen-related peptides (CRPs). **JAQ1**-treated mice were completely protected for at least 2 wk against lethal thromboembolism induced by infusion of a mixture of collagen (0.8 mg/kg) and epinephrine (60 mug/ml). The tail bleeding times in **JAQ1**-treated mice were only moderately increased compared with control mice probably because the treatment did not affect platelet activation by other agonists such as adenosine diphosphate or phorbol myristate acetate. These results suggest that **GPVI** might become a target for long-term prophylaxis of ischemic cardiovascular diseases and provide the first evidence that it is possible to specifically deplete an activating glycoprotein receptor from circulating platelets in vivo.

L5 ANSWER 6 OF 8 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.DUPLICATE 5
ACCESSION NUMBER: 2001:283535 BIOSIS
DOCUMENT NUMBER: PREV200100283535
TITLE: Evidence for two distinct epitopes within collagen for activation of murine platelets.
AUTHOR(S): Schulte, Valerie; Snell, Daniel; Bergmeier, Wolfgang; Zirngibl, Hubert; Watson, Steve P.; **Nieswandt, Bernhard (1)**
CORPORATE SOURCE: (1) Ferdinand-Sauerbruch Klinikum Wuppertal Universitaet Witten/Herdecke, Arrenbergerstr. 20, Haus 10, D-42117, Wuppertal: nieswand@klinikum-wuppertal.de Germany
SOURCE: Journal of Biological Chemistry, (January 5, 2001) Vol. 276, No. 1, pp. 364-368. print.
ISSN: 0021-9258.
DOCUMENT TYPE: Article
LANGUAGE: English
SUMMARY LANGUAGE: English
AB It has recently been shown that the monoclonal **antibody** **JAQ1** to murine glycoprotein VI (**GPVI**) can cause aggregation of mouse platelets upon **antibody** cross-linking and that collagen-induced platelet aggregation can be inhibited by preincubation of platelets with **JAQ1** in the absence of cross-linking (Nieswandt, B., Bergmeier, W., Schulte, V., Rackebrandt, K., Gessner, J. E., and Zirngibl, H. (2000) J. Biol. Chem. 275, 23998-24002). In the present study, we have shown that cross-linking of **GPVI** by **JAQ1** results in tyrosine phosphorylation of the same profile of proteins as that induced by collagen, including the Fc receptor (FcR) gamma-chain, Syk, LAT, SLP-76, and phospholipase Cgamma2. In contrast, platelet aggregation and tyrosine phosphorylation of these proteins were inhibited when mouse platelets were preincubated with **JAQ1** in the absence of cross-linking and were subsequently stimulated with a collagen-related peptide (CRP) that is specific for **GPVI** and low concentrations of collagen. However, at higher concentrations of collagen, but not CRP, aggregation of platelets and tyrosine phosphorylation of the above proteins (except for the adapter LAT) is re-established despite the presence of **JAQ1**. These observations suggest that a second activatory binding site, which is distinct from the CRP binding site on **GPVI** on mouse platelets, is occupied in the presence of high concentrations of collagen. Although this could be a second site on **GPVI** that is activated by a novel motif within the collagen molecule, the absence of LAT phosphorylation in response to collagen in the presence of **JAQ1** suggests that this is more likely to be caused by activation of a second receptor that is also coupled to the FcR gamma-chain. The possibility that this response is mediated by a receptor that is not coupled to FcR gamma-chain is excluded on the grounds that

aggregation is absent in platelets from FcR gamma-chain-deficient mice.

L5 ANSWER 7 OF 8 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.DUPLICATE 6
ACCESSION NUMBER: 2000:417229 BIOSIS
DOCUMENT NUMBER: PREV200000417229
TITLE: Expression and function of the mouse collagen receptor glycoprotein VI is strictly dependent on its association with the FcRgamma chain.
AUTHOR(S): **Nieswandt, Bernhard (1)**; Bergmeier, Wolfgang; Schulte, Valerie; Rackebrandt, Kirsten; Gessner, J. Engelbert; Zirngibl, Hubert
CORPORATE SOURCE: (1) IMMI, Klinikum Wuppertal, Universitaet Witten-Herdecke, Heusnerstrasse 40, D-42283, Wuppertal Germany
SOURCE: Journal of Biological Chemistry, (August 4, 2000) Vol. 275, No. 31, pp. 23998-24002. print.
ISSN: 0021-9258.
DOCUMENT TYPE: Article
LANGUAGE: English
SUMMARY LANGUAGE: English
AB Platelet glycoprotein (GP) VI has been proposed as the major collagen receptor for activation of human platelets. Human **GPVI** belongs to the immunoglobulin superfamily and is noncovalently associated with the FcRgamma chain that is involved in signaling through the receptor. In mice, similar mechanisms seem to exist as platelets from FcRgamma chain-deficient mice do not aggregate in response to collagen. However, the activating collagen receptor on mouse platelets has not been definitively identified. In the current study we examined the function and in vivo expression of **GPVI** in control and FcRgamma chain-deficient mice with the first monoclonal **antibody** against **GPVI** (**JAQ1**). On wild type platelets, **JAQ1** inhibited platelet aggregation induced by collagen but not PMA or thrombin. Cross-linking of bound **JAQ1**, on the other hand, induced aggregation of wild type but not FcRgamma chain-deficient platelets. **JAQ1** stained platelets and megakaryocytes from wild type but not FcRgamma chain-deficient mice. Furthermore, **JAQ1** recognized **GPVI** (approximately 60 kDa) in immunoprecipitation and Western blot experiments with wild type but not FcRgamma chain-deficient platelets. These results strongly suggest that **GPVI** is the collagen receptor responsible for platelet activation in mice and demonstrate that the association with the FcRgamma chain is critical for its expression and function.

L5 ANSWER 8 OF 8 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
ACCESSION NUMBER: 2001:311599 BIOSIS
DOCUMENT NUMBER: PREV200100311599
TITLE: Long-term antithrombotic protection by irreversible inactivation of platelet glycoprotein VI in mice.
AUTHOR(S): **Nieswandt, Bernhard (1)**; Schulte, Valerie (1); Bergmeier, Wolfgang (1); Mokhtari-Nejad, Rabee (1); Cazenave, Jean P.; Gachet, Christian; Zirngibl, Hubert (1)
CORPORATE SOURCE: (1) Molecular Oncology, Witten/Herdecke University, Wuppertal Germany
SOURCE: Blood, (November 16, 2000) Vol. 96, No. 11 Part 1, pp. 269a. print.
Meeting Info.: 42nd Annual Meeting of the American Society of Hematology San Francisco, California, USA December 01-05, 2000 American Society of Hematology
. ISSN: 0006-4971.
DOCUMENT TYPE: Conference
LANGUAGE: English
SUMMARY LANGUAGE: English
AB Coronary artery thrombosis is often initiated by abrupt disruption of the atherosclerotic plaque followed by deposition and activation of platelets on the subendothelial layers in the disrupted plaque. Because the

extracellular matrix protein collagen is the most thrombogenic constituent of the subendothelial layer, a selective inhibition of the collagen activation pathway in platelets may provide strong antithrombotic protection while preserving other platelet functions. Growing evidence suggests that platelet glycoprotein (GP) VI is the major collagen receptor for platelet activation making this receptor a good candidate for such a specific inhibition. In the current study, we have investigated the antithrombotic effects of the first monoclonal **antibody** (mAb) against mouse **GPVI** (**JAQ1**, Nieswandt et al; 2000, J Biol Chem, 275(31):23998-24002). Injection of 100 mug **JAQ1** only had mild and transient effects on platelet counts with a maximum drop of approximately 34 +- 7.4 % on day 1 and a return to normal after 2-3 days. **JAQ1** pretreated mice were completely protected against lethal thromboembolism induced by infusion of a mixture of collagen (0.8 mg/kg) and epinephrine (60 mug/kg) for at least two weeks (100% survivors on days 3, 7, and 14 after mAb injection, n=8 per group, 5% survivors in the control group, n=20). Aggregometric and flow cytometric studies demonstrated that platelets from **JAQ1** treated mice were completely resistant against activation with high concentrations of collagen (up to 50 mug/ml) and collagen related peptides (up to 100 mug/ml) ex vivo on days 3, 7, and 14. In **JAQ1** treated mice, **GPVI** was not detectable in a Western blot analysis of platelet lysates for minimally two weeks, suggesting irreversible inactivation (or degradation) of the receptor on circulating platelets. In contrast to collagen, other agonists, such as ADP or platelet aggregating agents, such as PMA induced normal activation and aggregation of these platelets. Consequently, the tail bleeding times were only moderately increased in **anti-GPVI** treated mice compared to control mice on day 3, 7, and 14. These results establish **GPVI** as an attractive target for long-term antithrombotic therapy.

=>